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Abstract

Title of thesis: **Study of senescence and possible mechanisms involved in arsenic-induced carcinogenesis in humans**

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Arsenic (As) induces various patho-physiological outcomes in humans like cancers including skin cancers, peripheral neuropathy (PN) and respiratory diseases. Though reports have shown that arsenic induced senescence (AIS) *in vitro*; population based studies on AIS and epigenetic regulation of AIS contributing to As-induced diseases remains unexplored. We investigated AIS, senescence-associated secretory phenotype (SASP) markers, telomere length alteration, epigenetic regulation involving altered senescence associated miRNA (SA-miRs) expression in arsenic exposed individuals with characteristic skin lesions and peripheral neuropathy. We also made an attempt to check the genetic damage, overall health status and telomere length in arsenic exposed children. Exposure assessment was done from drinking water and urine collected from arsenic exposed (N=120) and unexposed (N=60) individuals recruited from West Bengal, India. Senescence and telomere length alteration was evaluated using SA β-gal activity, ELISA and quantification of senescence proteins, alternative lengthening of telomere (ALT) associated proteins and telomerase activity. Relative telomere length (RTL) and SA-miRs in AIS was determined by qPCR. The downstream molecule of the miRNA associated with As-induced PN was quantified by immuoblotting. *In vitro* studies were conducted with sodium arsenite exposed HEK 293 cells, to revalidate the observations.

As-exposed individuals exhibited significantly increased senescent cells, upregulated senescence inducers, p53/p21 and SASP markers when compared to unexposed controls. As-exposed skin lesion group showed significantly increased RTL, which was telomerase-independent but exhibited an over-expression of ALT associated proteins. All the SA-miRs assessed were upregulated in the As-exposed compared to controls, specifically miR-29a. Further analysis found that highest expression of miR29a and peripheral myelin protein 22 (PMP22), a direct target of miR 29a, was among As-exposed individuals with PN. Analyzing other intermediate players regulating PMP22 expression revealed up-regulation of β-catenin and down-regulation of GSK-3β. Our findings suggest that up-regulation of β-catenin, possibly by miR29a mediated negative regulation of β-catenin inhibitors, may play a predominant role in expression of PMP22 which leads to formation of aggresomes. Further work to validate this mechanism is in process *in vitro*. Arsenic exposed children showed considerable genetic damage as measured by micronucleus assay in the three cell types and also adverse health outcomes like decreased haemoglobin content and gastritis. Telomere length of arsenic exposed children was slightly elevated though it did not reach the significance level.

Our findings suggest that arsenic exposure induces senescence *in vivo* and telomerase-independent elongation of telomere length is strictly associated with As-induced skin lesions in adults. Epigenetically, arsenic alters the expression of SA-miRs and the mir29a/beta catenin/PMP22 axis might be responsible for arsenic induced PN. However, in children, the telomere length increase and genetic damage in the three cell types and adverse health outcomes suggested that children are equally at danger of arsenic poisoning.